DATA EVALUATION RECORD
HONEY BEE - FIELD TESTING FOR POLLINATORS

i 141-5 (OPPTS 850. 3040)

1. CHEMICAL: Clothianidin PC Code No.: 044309

2. TEST MATERIAL: Clothianidin FS 600B G Purity: 595 g/L

3. CITATION:

Author: Liepold, K.

Title: Monitoring of potential effects of the drilling of clothianidin treated maize seeds on honeybees, guttation monitoring of maize seedlings under agronomic use conditions and assessment of the relevance of guttation for honeybees in Aquitaine (France).

Study Completion Date: January 6, 2010

Laboratory: Eurofins-GAB GmbH, Niefern, Oschelbronn, Germany

Sponsor: Bayer CropScience AG, Ecotoxicology, Monheim, Germany

Laboratory Report ID: S09-01405

DP Barcode: 374484

MRID No.: 47972303

4. REVIEWED BY: Moncie Wright, Staff Scientist, Cambridge Environmental, Inc.

Signature: Moncie V Wright **Date:** 08/01/11

APPROVED BY: Teri S. Myers, Ph.D., Senior Scientist, Cambridge Environmental Inc.

Signature: Teri S Myers **Date:** 08/01/11

5. APPROVED BY: Allen Vaughan, Biologist, ERB - V

Signature: **Date:**

6. DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the long-term toxicity of a pesticide to honey bees following an actual-use field exposure. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

7. STUDY PARAMETERS:

Scientific Name of Test Organism:	*Apis mellifera* L.
Age or Size of Test Organism at Test Initiation:	Queens in all colonies were of the same lineage and the bees in all colonies were young.
Definitive Study Duration:	126 days (6 day pre-exposure period before a 71-day exposure period followed by a 49-day post-exposure period).

8. CONCLUSIONS:

In a 126 day study (6 day pre-exposure period before a 71-day exposure period followed by a 49-day post-exposure period), the toxicity of dust from clothianidin-treated seed during drilling of treated maize seeds was examined in the honey bee, *Apis mellifera* L., under open field conditions at two test sites (the test fields were located near Bergerac) in the Aquitaine region of France. The treated site was planted with maize seeds dressed with the end-use product Clothianidin FS 600B G (AI: 595 g/L Clothianidin), and the other site was planted with untreated control seed. The treatment and the control plots were separated by 6.7 km. The maize seeds were sown at a nominal drilling rate of 2 units (100,000 seeds)/ha on May 6 (control) and May 7 (treatment), 2009.

Six honeybee colonies were placed at the edge of the each field plot at a distance of 1-2 m from the sowing area with the entrance facing the maize field. The colonies were established in a downwind position relative to the field in order to maximize potential dust exposure during drilling. The colonies were placed in the fields 6 days before drilling and remained at the study location for 65 days after seedling emergence. For the post exposure period the colonies were moved from the exposure plots to a monitoring location near Grenade sur Garonne, France (Haute Garonne). Throughout the study, colonies were assessed for mortality, colony strength, and brood and food store area. Additionally, the occurrence and duration of guttation, flight activity and bee behavior, and bees collecting guttation liquid were also observed.

Guttation was observed in both the morning and evening. Guttation on adjacent vegetation and on neighboring fields was observed on most days when guttation occurred on the control maize plot. No guttation was observed on adjacent vegetation around the treatment plot. The proportion of guttating plants varied from 0 to 100% of all plants in the respective assessed areas in both the control and treatment plots. Generally, the occurrence of guttation was more pronounced in the control plot compared to the treatment plot.

During the assessment of guttation in the control and treatment plots and in the 2 m^2 observation areas, similar numbers of bees were observed sitting on maize plants or on the ground. No honeybees were observed consuming guttation liquid or otherwise interacting with guttation liquid droplets in the control or treatment plot for the entire duration of the study period.

Flight activity was low in the morning due to low temperatures. Flight activity increased during the course of the day in both plots. The period of guttation and bee activity overlapped. Bee behavior in the front of the hives was normal in the both the treated and control plots for the entire exposure period.

The mean daily mortality during guttation was 10 bees/hive in the control group (May 16 to July 9, 2009; DAD 9-63) and 12.7 bees/hive in the treatment group (May 16 to July 17, 2009; DAD 9-71). The mean daily mortality for the entire exposure (71 days) was 11.6 and 13.3 bees/hive in the control and treated groups, respectively.

There were no biologically significant differences between the control and treatment group with regard to colony strength and brood/food area. However, the control group was severely compromised. Control colony C2 swarmed at the end of May, which resulted in a loss of the old queen and worker bees and nectar, and was not able to recover. Another colony of the control group, C4, had problems with queen egg laying activity, and the colony was reported dead at the beginning of August (after colonies were removed from the test site). The treatment colonies were also compromised, which likely contributed to the number of worker bees available per hive. Treatment Colony T2 showed symptoms of foulbrood at the beginning of August (after the colonies had been removed from the test site), and the colony moved out of the hive after treatment with Thymol. Hive T3 was unable to produce a new queen after the exposure period (July 23), and was without brood up to the end of the observation period. While there were no biologically significant differences, the data were likely invalid due to the severe issues noted by the study author and confirmed by the reviewer.

The reviewer's conclusions, based on visual assessments of the mortality and brood/food data, indicate that effects due to the test material would be indistinguishable from effects attributed to disease. The results of this study could be considered invalid due to the diseases and colony deaths that occurred in the control and treatment groups. Further, it is likely that the other 4 hives in each treatment and control group were also affected; therefore, the remaining available data may not be reliable for the purposes of assessing the toxicity of Clothianidin to honeybees. Finally, it is possible that the dust from the clothianidin-treated seeds weakened the colonies and made them more susceptible to the bacterial and fungal infections that occurred, or had a synergistic effect.

The reviewer concludes that the data presented in this study are inadequate to accurately determine the effects of clothianidin-treated maize seedlings on honeybees and colony health. Guttation fluid, dead honeybees and pollen and nectar from combs were not analyzed because the study authors determined there was no damage to individual bees or bee colonies due to clthianidin-treated maize exposure.

This study is scientifically sound and *satisfies/does not satisfy* the EFED concerning the guideline requirements for a field toxicity test with honeybees (Subdivision L, i 141-5 or 850.3040).

9. ADEQUACY OF THE STUDY:

A. Classification: Acceptable / Supplemental / Unacceptable

B. Rationale: N/A

C. Repairability: N/A

10. GUIDELINE DEVIATIONS: There were no guideline deviations.

11. SUBMISSION PURPOSE: This study was submitted to provide data on the toxicity of clothianidin to honeybees in a field test for the purpose of chemical reregistration.

Specifically, the test was conducted to determine the relevance of potentially occurring guttation in young maize plants in the Aquitaine region in France as a water source for honeybees, and to assess potential effects of Clothianidin residues from the seed treatment of the maize seeds in guttation liquid on bee colonies under field conditions. Additionally, assessments were performed on the potential effects of the maize drilling process during which the colonies might be exposed to Clothianidin-containing dust from the seed treatment.

12. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: **Species of concern (*Apis mellifera*, *Megachile rotundata*, or *Nomia melanderi*)**	*Apis mellifera* L. (Hymenoptera, Apidae)
Colony description at beginning of test:	Each colony occupied hives consisting of one box (consisting of a brood chamber and honeycomb box) that included 10 combs each.

Guideline Criteria	Reported Information
	Queens in all colonies were of the same lineage and approximately the same age. A queen excluder was placed between the brood chamber and honeycomb box to retain the queen in the brood chamber. There was 1 queen per colony and between 4,503 and 12,003 bees per hive at study initiation.
Pre-test health:	Bees were reportedly free of *Nosema* and *Varroa* disease symptoms.
Supplier	The colonies were supplied by a beekeeper, Mr. Chiapello, Mandelieu, France
All bees from the same source?	Yes

B. Test System

Guideline Criteria	Reported Information
Exposure Site Location and Establishment:	The test fields were located near Bergerac in the Aquitaine region of France. The treated and untreated maize seeds were sown on two different plots. The treated site was planted with clothianidin-dressed maize seed and the other planted with untreated control seed. The treatment and the control plots were separated by a distance of 6.7 km. The size of the field plots was *ca*. 2.2 ha for the treated plot and *ca*. 2.3 ha for the control. The maize seeds were sown at a nominal drilling rate of 2 units (100,000 seeds)/ha on

Guideline Criteria	Reported Information
	May 6 (control) and May 7 (treatment), 2009. Effective rates: Control: 99,900 seeds/ha Treatment: 101,100 seeds/ha
Site Preparation:	None reported.
Number of Replicates/Treatment:	Six colonies per field plot, with 1 treated and 1 control field plot
Post-exposure Site Location:	Near Grenade sur Garonne, France.
Lighting:	Natural; not further described.
Precipitation:	Precipitation measured during mortality assessments at the control plot ranged from *ca*. 0.0 to 55 L/m^2 during the exposure period (data obtained from Figure 23). The maximum rainfall event occurred between May 14 and 19, 2009 (38 L/m^2) and after July 13, 2009 (55 L/m^2).
Temperature:	Daily temperatures ranged from 6.4 to 36.6°C during the exposure period.
Relative humidity:	Mean relative humidity ranged from 28.0 to 100% during the exposure period.

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	None reported
Reference toxicant tested?	No
Duration of Exposure Period	71 days
Duration of Post-exposure Period	49 days in the monitoring site

Guideline Criteria	Reported Information
Test Substance(s):	Clothianidin FS 600B G Formulation Type: suspension Batch No.: PF90191228 AI: 595 g/L Clothianidin (analyzed)
Control Substance(s):	N/A- control seeds were not treated
Maize Seed:	Seed variety: PR38A24
Application Rate:	0.512 mg ai per seed (analyzed)
Verification of Application Rate:	Not reported
Method of Seed Coating:	Not reported
Colony Introduction:	The colonies were placed at the edge of the each field plot at a distance of 1-2 m from the sowing area with the entrance facing the maize field. The colonies were established in a downwind position relative to the field in order to maximize potential dust exposure during drilling. The colonies were placed in the fields 6 days before drilling and remained at the study location for 65 days after seedling emergence.

Guideline Criteria	Reported Information
Post-exposure:	The colonies were moved from the exposure plots to a monitoring location near Grenade sur Garonne, France (Haute Garonne).
Assessment scheme:	The part of the field plots that was considered to be most likely to be attractive to honeybees seeking water was assessed regarding the occurrence of guttation and/or dew (assessment area). The in-field assessment area (zones 1-4) covered a width of 5 m to the left and to the right from the outer bee hives at each field, and in length encompassed 58 parallel rows of maize (43.3 m) for the treatment and 55 rows (41.6 m) for the control. Each assessment started with zone 0 and ended with zone 4.
Assessment zones:	Zone 0 = off-field assessment area; between row number 1; 2-4 m away from the field. Zone 1 = rows 1-7; assessments were performed along each row; observers made assessments while walking. Zone 2 = rows 8-13; assessments were made for rows in groups of 3 (each 3rd row was a passing row). Zone 3 = rows 14-28; assessments were made for rows in groups of 5 (each 5th row was a passing row). Zone 4 = rows 29-58in the treatment plot and rows 29 to 55 in the control plot; assessments were made for rows in groups of 5 (each 10th row was a passing row). Additionally, there were six 2 m^2 plots that each covered 2 rows of maize seedlings.

D. Biological Assessments

Guideline Criteria	Reported Information

Guideline Criteria	Reported Information
Maize guttation:	The occurrence and proportion of maize plants displaying guttation and/or dew was monitored for 65 days after emergence, until no more guttation could be seen for 8 days in the control plot. This was determined by observers that walked through each passage row. The percentage was estimated at 10, 25, 50, 75, 90, and >90%. If less than 10% of the plants displayed guttation, the exact number of plants in an assessment row that showed guttation was counted. Guttation occurrence was checked in regular intervals from the early morning onwards until no more guttation droplets were visible. In addition, the general occurrence of guttation droplets was checked at each field at sunset of every day. One full observation period included the guttation assessments in the 4 established zones in the fields. Additionally, zone 0 was checked for the presence of guttation and/or dew on the off-field vegetation and to determine if the extent of guttation and/or dew on the off-field vegetation was more or less than that present on the plants in the maize field. If no guttation occurred at both field sites then the plants of neighboring fields or adjacent vegetation were checked for guttation.
Bees collecting guttation droplets:	After the assessment of guttation and honeybee activity in the zones the number of honeybees per assessment plot sitting on the ground or on plants, and the number taking up droplets was recorded during a 4 minute assessment period per plot. Any abnormal behavior was documented.

Guideline Criteria	Reported Information
Flight activity:	On each assessment day (those days on which guttation was observed), the flight activity at the hive entrance of each hive was documented at the start and end of each observation period. Flight activity was assessed by counting the number of bees entering the hive over 1 minute and by counting the number leaving the hive over 1 minute.
Mortality:	Linen sheets were spread on the ground in front of the hives and dead bee traps were attached to the entrance of each hive to measure mortality during the exposure period. Mortality was assessed three days before drilling, on the day of seeding (after seeding was done), and daily thereafter until the termination of the exposure phase. The dead bee traps were emptied daily at the same time of day and the bees were transferred within 10 hours into a deep freezer (≤-18°C) for potential residues analysis.

Guideline Criteria	Reported Information
Colony condition:	The condition of the colonies was recorded once before the hives were placed on the field plots and afterwards in weekly intervals during the exposure phase.
Brood:	During the monitoring phase the brood assessments were performed 6 times in weekly intervals. The following parameters were assessed: - Colony strength (number of bees) - Presence of a healthy queen (presence of eggs) - Pollen storage area and area with nectar or honey - Area containing cells with eggs, larvae, and capped cells The comb area covered with bees and cells with nectar, pollen, egg, larval, and capped cells was estimated per comb side and the total number of bees and cells containing the brood stages, pollen, and nectar on the comb was calculated. The mean values were calculated for each hive and assessment date.
Collection of guttation fluid:	Guttation fluid was sampled on days when sufficient guttation for sampling was available early in the morning in the treated plot. The samples were collected in the morning within the first hour of the assessments on the field outside the guttation assessment areas and in a distance of at least 20 m from the hives. The fluid was collected with plastic Pasteur pipettes and was stored in Eppendorf caps. Samples were stored on blue ice and transferred within 10 hours to a deep freezer (≤-18°C). During the trial, sampling occurred on 61 days.

Guideline Criteria	Reported Information
Collection of pollen and nectar from combs:	Samples of pollen and nectar were collected from the bee hive combs during each brood assessment after drilling during the exposure phase. If possible, one sample that weighed 1 gram was taken per colony in the control and treated plots. Each sample was taken from 3 different sections per hive, and then all 3 samples were pooled. Pieces of comb were cut from the comb using a clean knife for each sample. A spoon was used to collect nectar. Samples were stored cooled and transferred within 10 hours to a deep freezer (≤-18°C). No further preparation was performed because the residues were not analyzed.

E. Residue Analysis

Guideline Criteria	Reported Information
Guttation fluid, dead bees, pollen and nectar from combs:	The study author concluded that Clothianidin-treated maize did not have negative effects on any of the biological endpoints measured; therefore, the author deemed it unnecessary to perform residue analysis.

13. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Signed and dated No Data Confidentiality, GLP, and Quality Assurance Statements were provided. This study was conducted in compliance with the most recent edition of the Principles of Good Laboratory Practice, Chemikaliengesetz, Attachment 1, Germany, and the OECD Principles of Good Laboratory Practice. The German

Guideline Criteria	Reported Information
	requirements are based on the OECD Principles of GLP, which are accepted by regulatory authorities throughout the European Community, the United States of America (FDA and EPA) and Japan (MHW, MAFF, and METI) on the basis of intergovernmental agreements. This study was not conducted according to any established guidelines; therefore, it was performed according to the study plan and SOPs of eurofins-GAB.
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Observations of guttation and proportion of guttating plants:
Guttation was observed in both the morning and evening. Guttation was observed for a total of 40 days in the control plot and for 62 days in the treatment plot during the exposure period (assessments began the first day after emergence until removal of the hives from the exposure plot). However, assessments were only carried out for 39 days in the control plot and for 60 days in the treated plot due to unfavorable weather conditions. When guttation did occur, there were totals of 129 assessments in the control plot and 117 assessments in the treatment plot.

Guttation on adjacent vegetation and on neighboring fields was observed on most days when guttation occurred on the control maize plot. No guttation was observed on adjacent vegetation around the treatment plot.

The proportion of guttating plants varied from 0 to 100% of all plants in the respective assessed areas in both the control and treatment plots. In general, guttation occurred at a similar rate over the 4 zones assessed, but not at a similar rate between the control and treatment. Generally, the occurrence of guttation was more pronounced in the control plot compared to the treatment plot. Dew and guttation did not occur together on all assessment days. Generally, there were more days with occurrences of guttation only as compared to days with both guttation and dew.

Honeybees visiting plants displaying guttation:

During the assessment of guttation in the control plot, bees were observed sitting on maize plants or on the ground in 15 out of 129 assessments (1-5 bees per assessment). In the 2 m^2 observation areas, bees were found sitting on the ground or on plants on 5 out of 127 assessments (1 single bee per area).

In the treated plot, bees were observed sitting on plants or on the ground or flying over the crop in 14 of the 117 assessments (1-2 bees per assessment). In the 2 m^2 areas, bees were on the ground or on plants for 12 out of 111 assessments (one single bee per area).

No honeybees were observed consuming guttation liquid or otherwise interacting with guttation liquid droplets in the control or treatment plot for the entire duration of the study period.

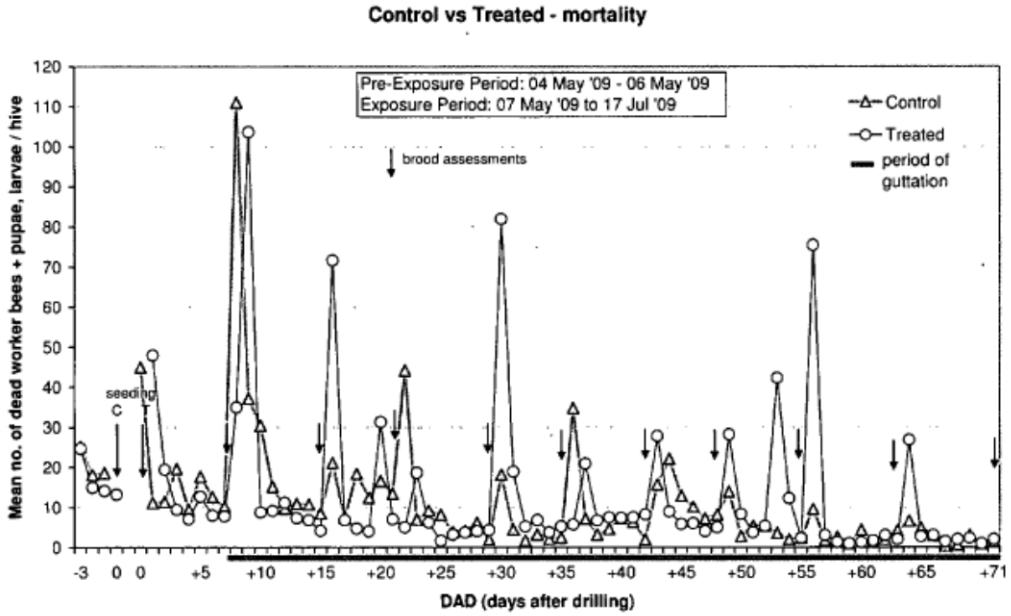
Flight activity:
Flight activity was low in the morning due to low temperatures. Flight activity increased during the course of the day in both plots. The period of guttation and bee activity overlapped. Bee behavior in the front of the hives was normal in the both the treated and control plots for the entire exposure period.

Mortality:
The daily mean pre-exposure (DAD -3 to -1) mortality (linen sheets + dead bee traps) in the control and treatment groups was 20.7 and 17.9 bees/hive, respectively. Drilling in the control plot was performed one day before drilling in the treatment plot. The pre- and post-exposure phases were adapted to the time schedule in the treatment plot. One day later, mortality in the control field was 45 bees/hive as compared to 48 bees/hive in the treated field. On the second day after drilling (DAD +2), mortality increased again in both the control and treated plot.

The mean daily mortality both the control and treatment group demonstrated the same tendency to fluctuate and increase and decrease (Figure 1). Mortality peaks tended to occur simultaneously in both the control and treatment group. Increases in the number of dead bees in front of the hives tended to occur after the brood assessments, which cause colony disturbance. During the exposure phase, the mortality in front of the hives was similar between the control and treated group. Toward the end of the exposure phase, the impact of the assessments was larger in the treatment plot.

The mean daily mortality during guttation was 10 bees/hive in the control group (May 16 to July 9, 2009; DAD 9-63) and 12.7 bees/hive in the treatment group (May 16 to July 17, 2009; DAD 9-71). The mean daily mortality for the entire exposure (71 days) was 11.6 and 13.3 bees/hive in the control and treated groups, respectively.

Figure 1. *Mean number of dead worker bees, pupae, and larvae/hive/day collected in the dead bee traps and on the linen sheet in front of the hives in the control and treatment groups before and during the time of exposure at the field site.*



Colony condition and brood development:

At the first brood assessment, colony strength (=mean number of bees/hive) in the control hives ranged from 4,503 to 11,002 bees. Colony strength in the treatment hives ranged from 5,380 to 12,003 bees. Only the bees that were present in the hives at the time of the assessment were included in the estimates. A portion of the worker bees was outside foraging, so the estimates underestimate actual colony strength. The mean number of bees in the treatment plot was on a higher or similar level as the control during the entire test period (Figure 2). By the end of the observation period in autumn, colony strength decreased because of natural decreases in the breeding activity of the bee colonies before winter. The September assessment yielded colony strength in the control hives ranging from 3,256 to 3,945 and strength in the treatment hives ranging from 882 to 5,070 bees.

The development of the mean amount of brood on the combs (eggs, larvae, and pupae) and food stores (nectar and pollen) in the treatment group showed a stronger increase during the observation period as compared to the controls (Figures 3 and 4).

The mean amount of brood in the treatment plots decreased between the assessment before colony set-up in the fields and the first assessment after set-up. After the first assessment after set-up the colonies increased the mean amount of brood on the combs. From mid-June to the end of the observation period (September 3, 2009) the amount of brood decreased again in both the

control and treatment groups because of a natural decrease in the breeding activity due to the time of year and a lower amount of natural food sources before winter.

In both the control and treatment groups, five out of six colonies exhibited a reduced abundance of brood stages at different times during the assessment period from the end of April to the beginning of September. On the assessment during mid-May, control colony C3 exhibited a lack of eggs and larvae, but the colony was able to recover. Colonies C1 and C6 exhibited a lack of brood starting at the end of May. In colony C1, a new queen was added June 5, 2009, and all brood stages were present again starting June 18, 2009. In colony C6, the dead queen was found in the dead bee trap, attached in front of the hive. The colony produced a new fertile queen that laid her first eggs on July 17, 2009. Control colony C2 swarmed at the end of May, which resulted in a loss of the old queen and worker bees and nectar, and was not able to recover. Another colony of the control group, C4, had problems with queen egg laying activity, and the colony was reported dead at the beginning of August.

In the treatment group, colony T5 demonstrated a lack of brood at the beginning of the study and at different assessments; the queen was replaced by mid-June. This queen did not survive, but the colony produced a new fertile queen that started to lay eggs. All brood stages were present at the beginning of August. Treatment colonies T1 and T3 exhibited a lack of eggs and/or larvae at mid-May. Hive T1 recovered, but T3 was unable to produce a new queen, and was without brood up to the end of the observation period. In colony T6, no eggs were observed in mid-June, but the colony was able to produce a new fertile queen that started to lay eggs on July 17, 2009. Colony T2 showed symptoms of foulbrood at the beginning of August, and the colony moved out of the hive after treatment with Thymol.

Symptoms of chalkbrood and Varroa infection/infestation were observed in the both the control and treatment groups. These infections are likely the reason for the problems observed in the colonies. A treatment-related effect was not observed for any of the assessment endpoints.

Figure 2. *Mean number of honeybees per hive (=colony strength) in the control and treatment group.*

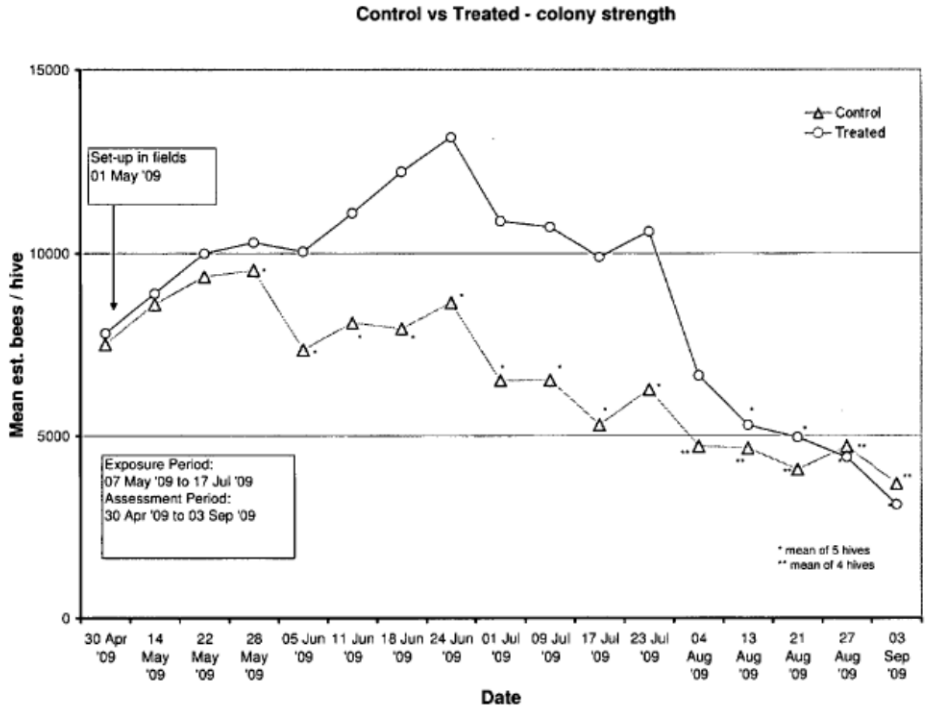


Figure 3. *Mean comb area per hive (%) covered with brood cells (eggs, larvae, and pupae) and with food stores (nectar and pollen) in the treatment group.*

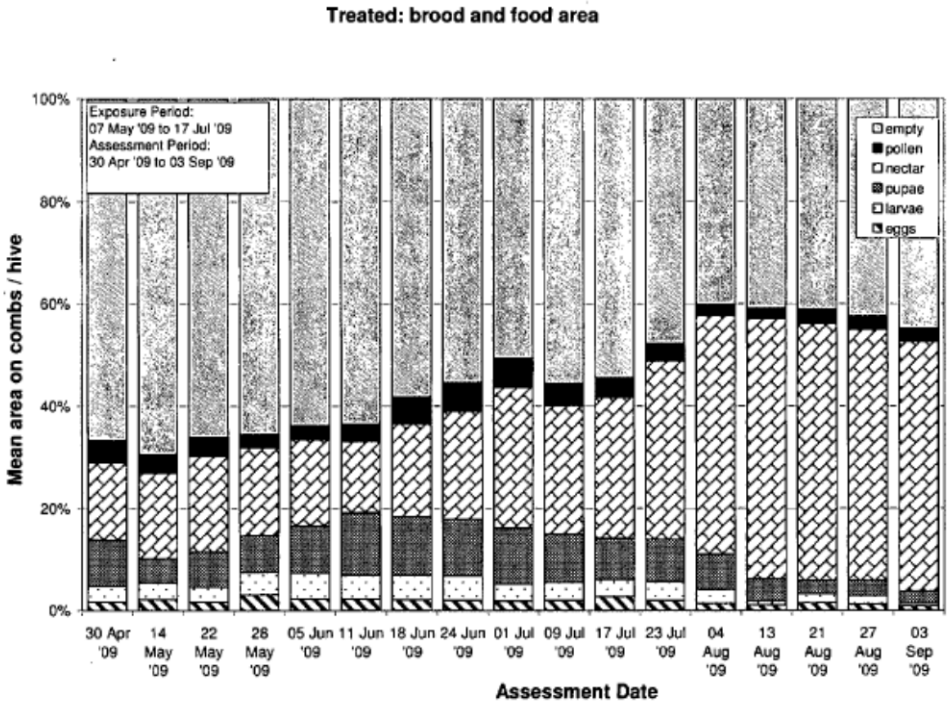
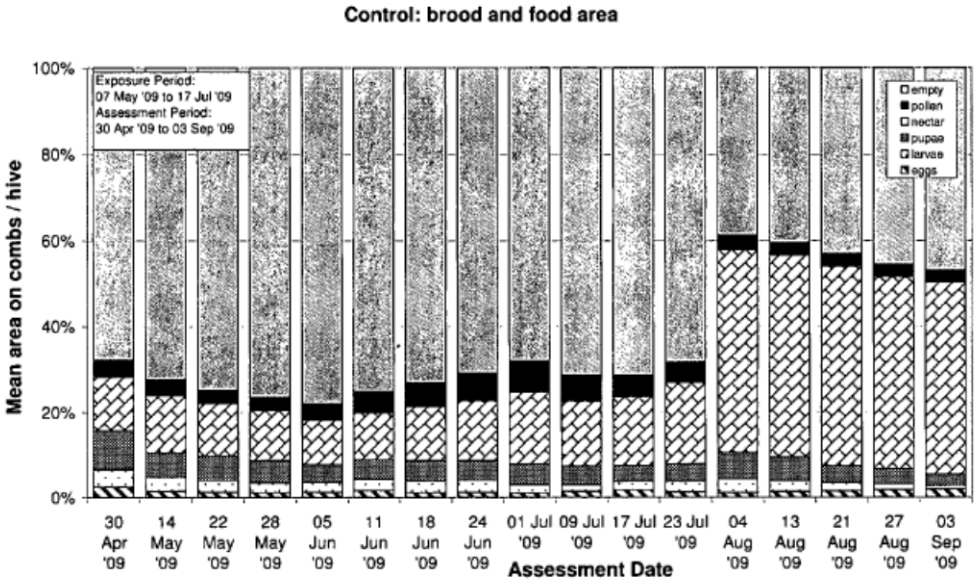


Figure 4. *Mean comb area per hive (%) covered with brood cells (eggs, larvae, and pupae) and with food stores (nectar and pollen) in the control group.*



Reported Statistical Results:

The study author did not perform statistical analysis on any of the parameters measured.

14. REVIEWER'S VERIFICATION OF STATISTICAL RESULTS:

Replicate data were provided for the bee trap mortality data when considering each individual hive as a replicate. However, individual hive data was not provided for the mortality data obtained from linen sheets placed in front of each hive. There were low average mortalities recorded on the linen sheets of the treatment group for both the exposure during guttation period (9 to 63/71 DAD) and the entire exposure period (0-71 DAD). Total mortalities (bee traps + linen sheets) were low and comparable between the control and treatment groups. For the period of exposure during guttation, total mortality averaged 10 and 12.7 bees/hive in the control and treatment groups, respectively. For the total period of exposure, mortality averaged 11.6 and 13.3 bees/hive in the control and treatment groups, respectively.

There was a single day where there were possibly biologically significant differences between the control and treatment group (May 16, 2009); however, there were a larger number of assessments where the control mortality was much higher than the treatment mortality, and the reviewer could not discern whether this was a natural fluctuation. Further, based on the major issues observed in the control and treatment groups (inability to recover from loss of a queen after queen death, chalkbrood, foulbrood, and colony mortality), the remaining available data is likely not reliable.

The reviewer visually verified the reported results for colony strength and brood/food area, and determined that there were no biologically significant differences between the control and treatment group. However, the control group was severely compromised. Control colony C2 swarmed at the end of May, which resulted in a loss of the old queen and worker bees and nectar, and was not able to recover. Another colony of the control group, C4, had problems with queen egg laying activity, and the colony was reported dead at the beginning of August (after colonies were removed from the test site). The treatment colonies were also compromised, which likely contributed to the number of worker bees available per hive. Treatment Colony T2 showed symptoms of foulbrood at the beginning of August (after the colonies had been removed from the test site), and the colony moved out of the hive after treatment with Thymol. Hive T3 was unable to produce a new queen after the exposure period (July 23), and was without brood up to the end of the observation period. While there were no biologically significant differences, the data were likely invalid due to the severe issues noted by the study author and confirmed by the reviewer.

16. REVIEWER'S COMMENTS:

The reviewer's conclusions were not in complete agreement with the study author's. The reviewer and the study author's results were in agreement with regard to a lack of statistical significance; however, the study author did not adequately consider confounding effects due to severe disease and mortality that plagued the hives in both the control and treatment groups. Two colonies each from the control and from the treatment group were unable to recover from symptoms of chalkbrood and Varroa infection/infestation that were observed in the both the control and treatment groups. Control colony C2 swarmed at the end of May, which resulted in a loss of the old queen and worker bees and nectar, and was not able to recover. Another colony of the control group, C4, had problems with queen egg laying activity, and the colony was reported dead at the beginning of August (after colonies were removed from the test site). Treatment Colony T2 showed symptoms of foulbrood at the beginning of August (after the colonies had been removed from the test site), and the colony moved out of the hive after treatment with Thymol. Hive T3 was unable to produce a new queen after the exposure period (July 23), and was without brood up to the end of the observation period. The study author concluded that a treatment-related effect was not observed for any of the assessment endpoints. The reviewer's conclusions, based on visual assessments of the mortality and brood/food data, indicate that any effects due to the test material would be indistinguishable from effects attributed to disease. The results of this study could be considered invalid due to the diseases and colony deaths that occurred in the control and treatment groups. Further, it is likely that the other 4 hives in each treatment and control group were also affected; therefore, the remaining available data may not be reliable for the purposes of assessing the toxicity of Clothianidin to honeybees. Finally, it is possible that the dust from the clothianidin-treated seeds weakened the colonies and made them more susceptible to the bacterial and fungal infections that occurred, or had a synergistic effect.

Climatic data (temperature, humidity, rainfall, and cloud formation) were recorded at the control field plot. Temperature and humidity were recorded at 15 minute intervals using a data logger starting May 4, 2009 and ending July 17, 2009. Daily rainfall was measured using a rain gauge. While colonies were located at the monitoring location, weather data were collected from the nearby official government weather station in Villematier.

Soil samples were collected from the test fields for determination of physico-chemical properties. Five soil cores (5 cm width) were collected to a depth of 20 cm from each corner of the treated and control field plot (4 x 5 samples per field). Standard soil parameters were determined:

Table 6: Soil characterisation

	Control	Treatment
Soil Type[5)]	High loamy sand	High loamy sand
pH value ($CaCl_2$) [1)]	7.6	7.1
WHC_{max} [g /100 g soil dry weight] [2)]	39.1	43.5
TOC [%] [3)]	2.04	2.63
Clay [%] (< 0.002 mm) [4)]	13.4	17.0
Silt [%] (0.063 mm to ≥ 0.002 mm) [4)]	32.8	33.1
Sand [%] (2 mm to ≥ 0.063 mm) [4)]	53.8	49.9

WHC_{max} = Maximum Water Holding Capacity
TOC = Total Organic Carbon
[1)]DIN ISO 10390 mod
[2)]Schaller 1993
[3)]DIN ISO 10694
[4)]DIN 19683
[5)]DIN 4220

16. REFERENCES:

DIN 19683 BLATT 3 (1973-04): Physikalische Laboruntersuchungen – Bestimmung der Korngrossenzusammensetzung.

DIN 4220 (2008-11) Bodenkundliche Standortbeurteilung – Kennzeichnung, Klassifizierung und Ableitung von Bodenkennwerten.

DIN ISO 10390 (2005-12): Bodenbeschaffenheit – Bestimmung des pH-Wertes.

DIN ISO 10694 (1996-08): Bodenbeschaffenheit – Bestimmung von organischem Kohlenstoff und Gesamtkohlenstoff nach trockener Verbrennung.

Imdorf, A. and Gerig, L. (1999): Lehrgang zur Erfassung der Volksstarke, Schweizerisches Zentrum fur Bienenforschung.

Imdorf, A.; Buehlmann, G.; Gerig, L.; Kilchmann, V. and Wille, H. (1987): Uberprufung der Schatzmethode zur Ermittlung der Brutflache und der Anzahl Arbeiterinnen in freifliegenden Bienenvolkern, Apidologie 18 (2), 137-146.

Schaller, K. (1993): Praktikum zur Bodenkunde und Pflanzenernahrung, Geisenheimer Berichte Band 2.